DATA EVALUATION RECORD

DICAMBA

Study Type: OCSPP Non-Guideline; Histopathological Follow-Up Study in Rats

EPA Contract No. EP-W-16-018
Task Assignment No. 34-3-001 (MRID 51129102)

Prepared for
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This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. Contractor's role did not include establishing Agency policy.

EPA Reviewer: Sarah Dobreniecki **Signature:** Sarah Dobreniecki
Risk Assessment Branch VII, HED (7509P) **Date:** 9/16/2020
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DATA EVALUATION RECORD

STUDY TYPE: Histopathological Follow-Up Study in Rats; OCSPP Non-Guideline; OECD 489.

PC CODE: 029801

DP BARCODE: D458715

TXR #: 0058082

TEST MATERIAL (PURITY): Dicamba (89.8% a.i.)

SYNONYMS: SAN837; Dicamba technical; 3,6-dichloro-2-methoxybenzoic acid

CITATION: Herring, T. (2019) Dicamba – Crl:CD(SD) rat histopathological follow-up study. Envigo CRS Ltd., Alconbury, Huntingdon, Cambridgeshire, UK. Laboratory Study No.: NS52VW, February 15, 2019. MRID 51129102. Unpublished.

SPONSORS: Syngenta, Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire, UK

BASF Corp., 100 Park Avenue, Florham Park, NJ

EXECUTIVE SUMMARY: In a concurrently-reviewed, non-guideline, *in vivo* comet test (MRID 51129101), increased DNA strand breaks accompanied by increased numbers of hedgehog cells were observed in the duodenum of Crl:CD(SD) male rats administered dicamba in aqueous 0.5% methylcellulose via oral gavage (dose volume 10 mL/kg) at dose levels of 37.5 or 75 mg/kg/day. The present study was performed in order to further investigate the effects on point-of-contact tissues. In this non-guideline, histopathological follow-up study (MRID 51129102), groups of five Crl:CD(SD) male rats were administered dicamba (89.8% a.i., batch # P.MG2726410) in aqueous 0.5% methylcellulose via oral gavage (dose volume 10 mL/kg) at dose levels of 0, 37.5, or 75 mg/kg/day; two doses were administered approximately 24 hours apart. At approximately 2, 6, 24, and 48 hours after the second dose, the rats were euthanized, and sections of the stomach and duodenum were excised, fixed, and routinely processed for microscopic pathological examinations. Sections were visualized with hematoxylin and eosin or by using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and active caspase 3 stains to detect cytotoxicity, necrosis, and/or apoptosis.

There was no treatment-related cytotoxicity, necrosis, or apoptosis at the dose levels administered in this study.

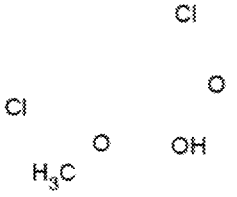
This study is classified as **acceptable / non-guideline**.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Dicamba

Description:	White solid
Batch #:	P.MG2726410
Purity:	89.8% a.i.
CAS # of TGAI:	1918-00-9
Stability:	Approximately two years stored at <30°C
Structure:	

2. Vehicle: Aqueous 0.5% (w/v) methylcellulose (batch #: MKBN2740V; Sigma).

3. Test animals

Species:	Rat (male only)
Strain:	Crl:CD(SD)
Age / weight at Day 1:	Approximately 7-8 weeks / 206-266 g
Source:	Charles River UK Ltd. (Margate, Kent, England)
Housing:	It was stated the groups were kept in cages; however, additional information (type of cage and rats per cage) were not provided. Rats were provided untreated wood chew blocks and a red plastic shelter for environmental enrichment.
Diet:	Pelleted Envigo Teklad 2014C diet, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	20-24°C
Humidity:	40-70%
Air changes:	Not provided
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	5 days minimum

B. STUDY DESIGN

1. In-life dates: Not reported

2. Animal assignment: Following receipt, the rats were weighed and randomly assigned to the groups in Table 1. No additional information was reported.

TABLE 1: Study design [a]							
Group	Dose (mg/kg/day)	Concentration (mg/mL)	Total # males	Time of tissue sampling (hours)			
				2	6	24	48
1	0	0	20	5	5	5	5
2	37.5	3.75	20	5	5	5	5
3	75	7.5	20	5	5	5	5

a Data were obtained from pages 20 and 52 of MRID 51129102.

3. **Dose-selection rationale:** The doses were the same as those used in the comet test (MRID 51129101).

4. **Preparation of test formulations:** For each dose formulation, an appropriate amount of the test substance (adjusted for purity) was weighed and ground in a mortar with a pestle; vehicle (aqueous 0.5% w/v methylcellulose) was added and mixed to form a paste. Additional vehicle was added to produce a smooth, pourable suspension, and the suspension was brought up to final volume with vehicle and homogenized.

   Homogeneity and stability analyses were reported in MRID 51129101; the results are reported here. Concentration analyses were performed on freshly prepared suspensions; single samples from the top, middle, and bottom (vehicle was sampled twice from the middle) were analyzed.

   Results

   Homogeneity analyses (%CV): 0.17-4.18%

   Stability analysis (% of time 0): 100.7-107.1% following one day storage at ambient temperatures; 101.7-106.6% following eight days storage refrigerated.

   Concentration analysis (% of nominal): 96.3-99.3%

   The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Dose administration:** The dose suspensions and positive control were administered by oral gavage at a dose volume of 10 mL/kg; the volumes were calculated from the most recent body weights. The dose suspensions were administered twice approximately 24 hours apart.

6. **Statistics:** Statistical analyses were not reported. Because only histopathological results were investigated, the Reviewers do not consider a lack of statistical analyses to be a major deficiency.

C. METHODS

1. **Observation:** The rats were observed regularly throughout the working day for mortality and morbidity.

2. **Body weight:** All rats were weighed following arrival, on each day of dosing, and at euthanasia. Rats having tissue sampling at 48 hours after the second dose were weighed on Day 3.

3. **Food consumption:** Food consumption was not reported.

4. **Microscopic pathology:** At approximately 2, 6, 24, or 48 hours after administration of the second dose, the rats were euthanized by carbon dioxide asphyxiation. The stomach and duodenum were excised, and sections were fixed in 10% neutral-buffered formalin, routinely processed, and visualized with hematoxylin and eosin or by using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) or active caspase 3 stains (methods for the TUNEL and active caspase stains were not reported). All tissues and time points were examined microscopically for signs of cytotoxicity, necrosis, and apoptosis.

II. RESULTS

A. CLINICAL SIGNS OF TOXICITY: There were no clinical signs of toxicity observed in any group. All rats survived to scheduled euthanasia.

B. BODY WEIGHTS: Body weight data are presented in Tables 2a-2c. In the 2- and 6-hour sampling groups, all rats in all treatment groups (except one 37.5 mg/kg/day rat; 2-hour sampling time) lost minor amounts of weight. These losses did not affect the results of the study. The 24- and 48-hour sampling groups were unaffected by treatment.

TABLE 2a. Mean (± SD) body weights (g) in rats administered two doses of dicamba via oral gavage 24 hours apart and euthanized at 2 and 6 hours after the second dose. [a]

Dose (mg/kg/day)	Day 1	Day 2	Day 2 euthanasia
2-hour sampling time			
0	240 ± 13.8	245 ± 13.9	242 ± 14.9
37.5	239 ± 12.1	243 ± 12.1	239 ± 10.6
75	234 ± 17.0	239 ± 18.7	234 ± 17.9
6-hour sampling time			
0	226 ± 7.1	231 ± 7.4	222 ± 6.2
37.5	229 ± 6.5	234 ± 7.9	225 ± 5.5
75	233 ± 22.5	235 ± 24.3	229 ± 25.8

a Data were obtained from Appendix 1 on page 27 of MRID 51129102. N = 5.

TABLE 2b. Body weights (g) in rats administered two doses of dicamba via oral gavage 24 hours apart and euthanized at 24 hours after the second dose. [a]

Dose (mg/kg/day)	Day 1	Day 2	Day 3 euthanasia
0	231 ± 14.0	237 ± 11.4	241 ± 14.0
37.5	245 ± 20.1	251 ± 20.5	256 ± 21.3
75	233 ± 17.5	238 ± 18.8	244 ±17.3

a Data were obtained from Appendix 1 on page 28 of MRID 51129102. N = 5.

TABLE 2c. Body weights (g) in rats administered two doses of dicamba via oral gavage 24 hours apart and euthanized at 48 hours after the second dose. [a]				
Dose (mg/kg/day)	**Day 1**	**Day 2**	**Day 3**	**Day 4 euthanasia**
0	224 ± 11.3	230 ± 12.2	236 ± 13.1	244 ± 12.6
37.5	245 ± 8.7	250 ± 6.4	259 ± 10.2	268 ± 9.6
75	251 ± 11.0	257 ± 10.6	265 ± 12.4	276 ± 12.8

a Data were obtained from Appendix 1 on page 29 of MRID 51129102. N = 5.

C. MICROSCOPIC PATHOLOGY:

1. **Stomach:** It was stated that there was no increase in the number/proportion of apoptotic/necrotic cells in any animal on the hematoxylin and eosin stained sections; further, no lesions of any kind were noted.

   Caspase 3 staining revealed minimal staining of scattered cells in the limiting ridge or glandular stomach in several treated and control rats with no apparent relationship to dose or time after dosing.

   Minimal to slight TUNEL staining was seen in the non-glandular region of the stomach in several treated and control rats. There was no apparent relationship to dose or time after dosing, and these findings were considered artefactual as there was no accompanying caspase 3 staining.

2. **Duodenum:** It was stated that there was no increase in the number/proportion of apoptotic/necrotic cells in any animal on the hematoxylin and eosin stained sections; further, no lesions of any kind were noted.

   It was stated that caspase 3 staining revealed minimal to slight numbers of scattered apoptotic cells throughout the length of the villi in all animals with the number and distribution of these cells having no apparent relationship to dose or time after dosing.

   It was stated that there was no TUNEL staining observed in the duodenum in any animal.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: There was no detectable increase in apoptotic/necrotic cells in the stomach or duodenum related to treatment with dicamba.

B. REVIEWER COMMENTS: The Reviewers agree with the Investigators' Conclusions. Treatment with dicamba did not cause cytotoxicity, necrosis, or apoptosis at the dose levels administered in this study.

This study is classified as **acceptable / non-guideline**.

C. STUDY DEFICIENCIES: The following deficiencies were noted:

- Methods for the TUNEL and caspase 3 staining procedures were not reported.
- Tabular data (including severity grading) for the microscopic findings were not provided.